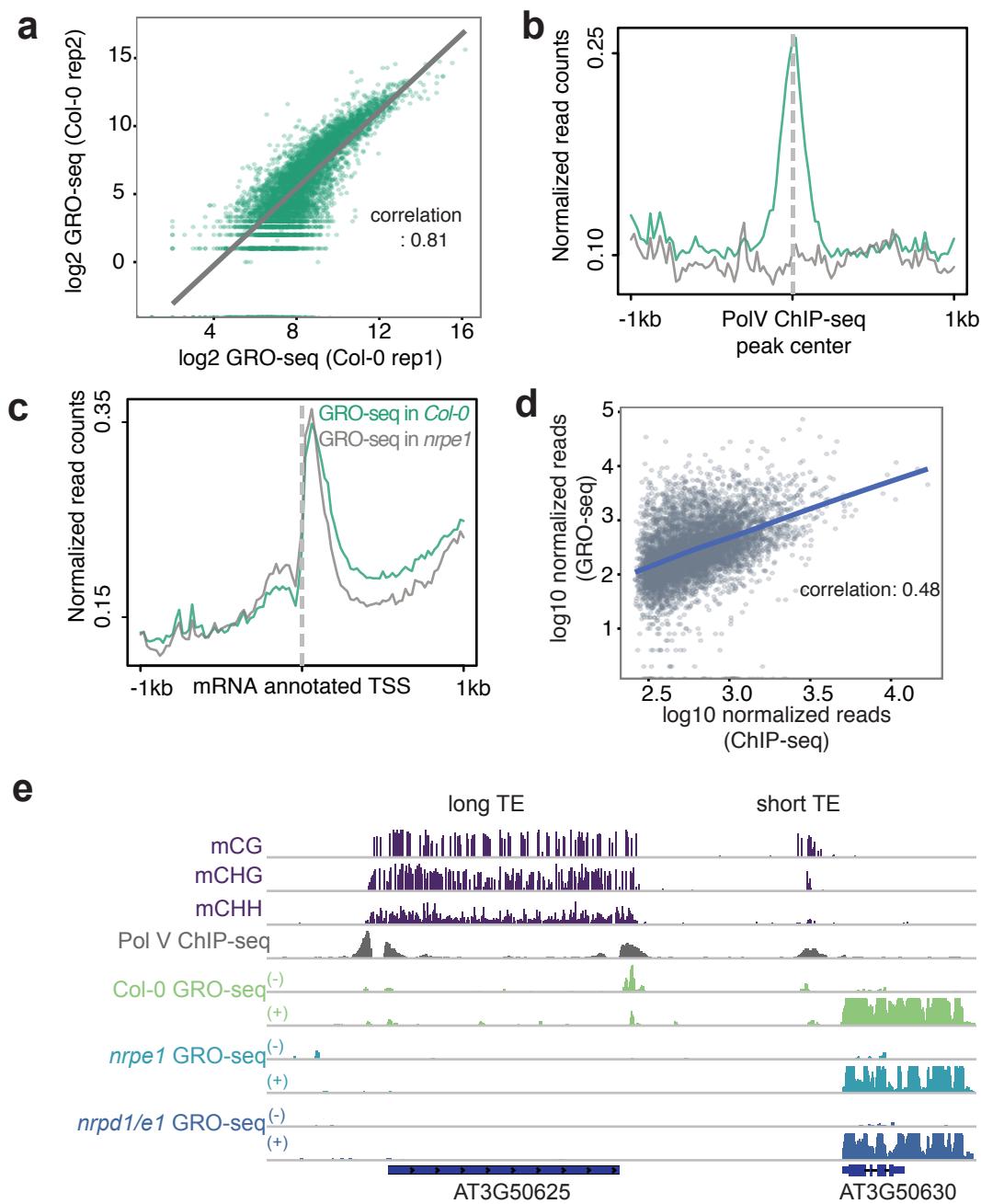
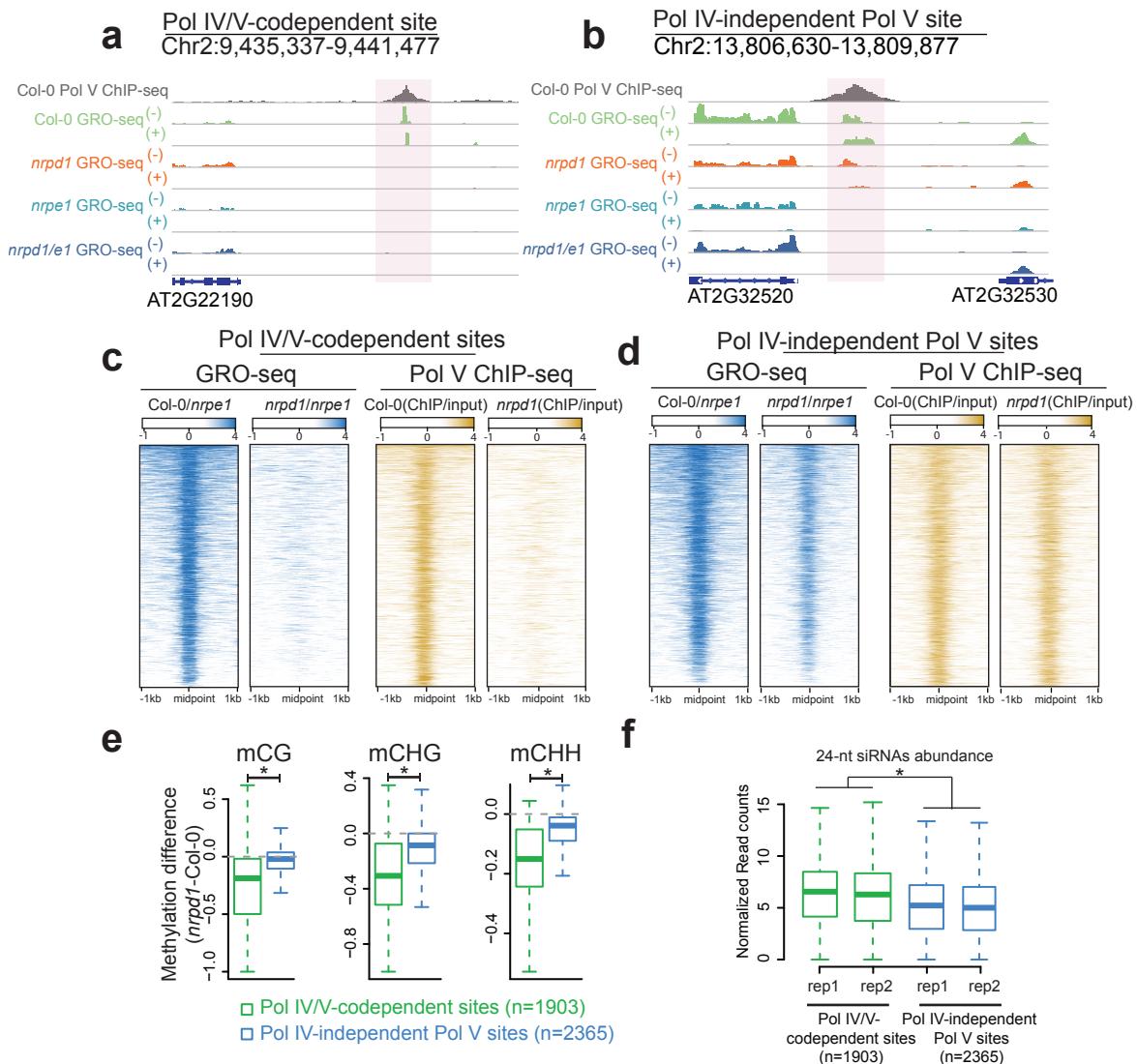


## Supplementary Fig.1



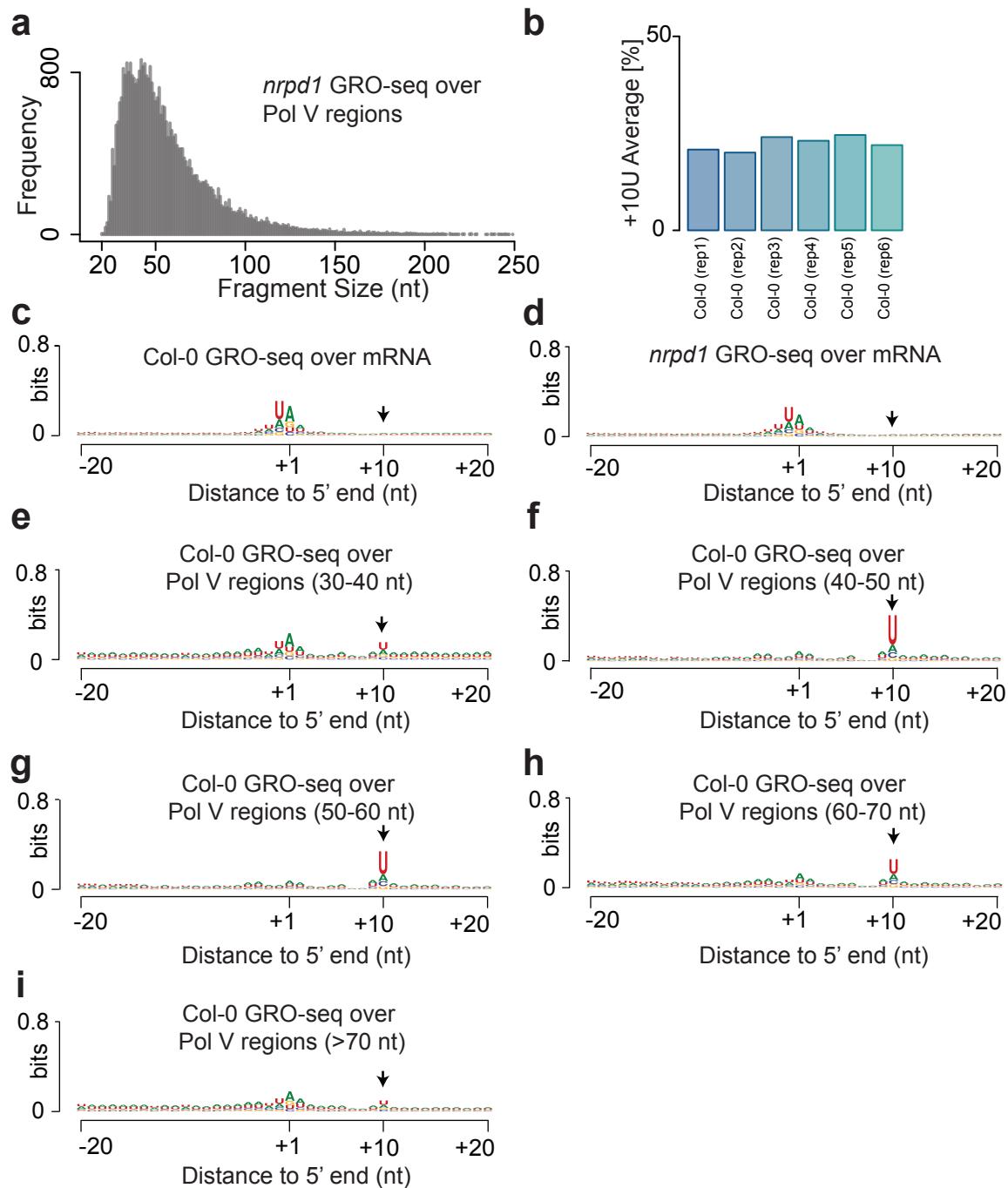
**Supplementary Figure 1.** Modified GRO-seq is able to capture nascent Pol V-dependent transcripts. **a**, Scatterplot of signals from two independent GRO-seq experiments in Col-0. The Pearson's correlation coefficient is calculated and shown on the plot. **b**, Metaplot showing GRO-seq signals over Pol V-occupied regions in Col-0 and *nrpe1*. **c**, Metaplot showing GRO-seq signals over annotated genes in Col-0 and *nrpe1*. **d**, Scatterplot of normalized signals from Pol V ChIP-seq versus GRO-seq in Col-0. The Pearson's correlation coefficient are calculated and shown on the plot. **e**, Genome browser screenshot for CG, CHG, and CHH methylation in Col-0, Pol V ChIP-seq signals in Col-0, and GRO-seq signals in Col-0, *nrpe1*, and *nrpd1/e1* of a representative long TE and a representative short TE. Plus (+) and Minus (-) indicate the strandness of GRO-seq signal.

## Supplementary Fig.2



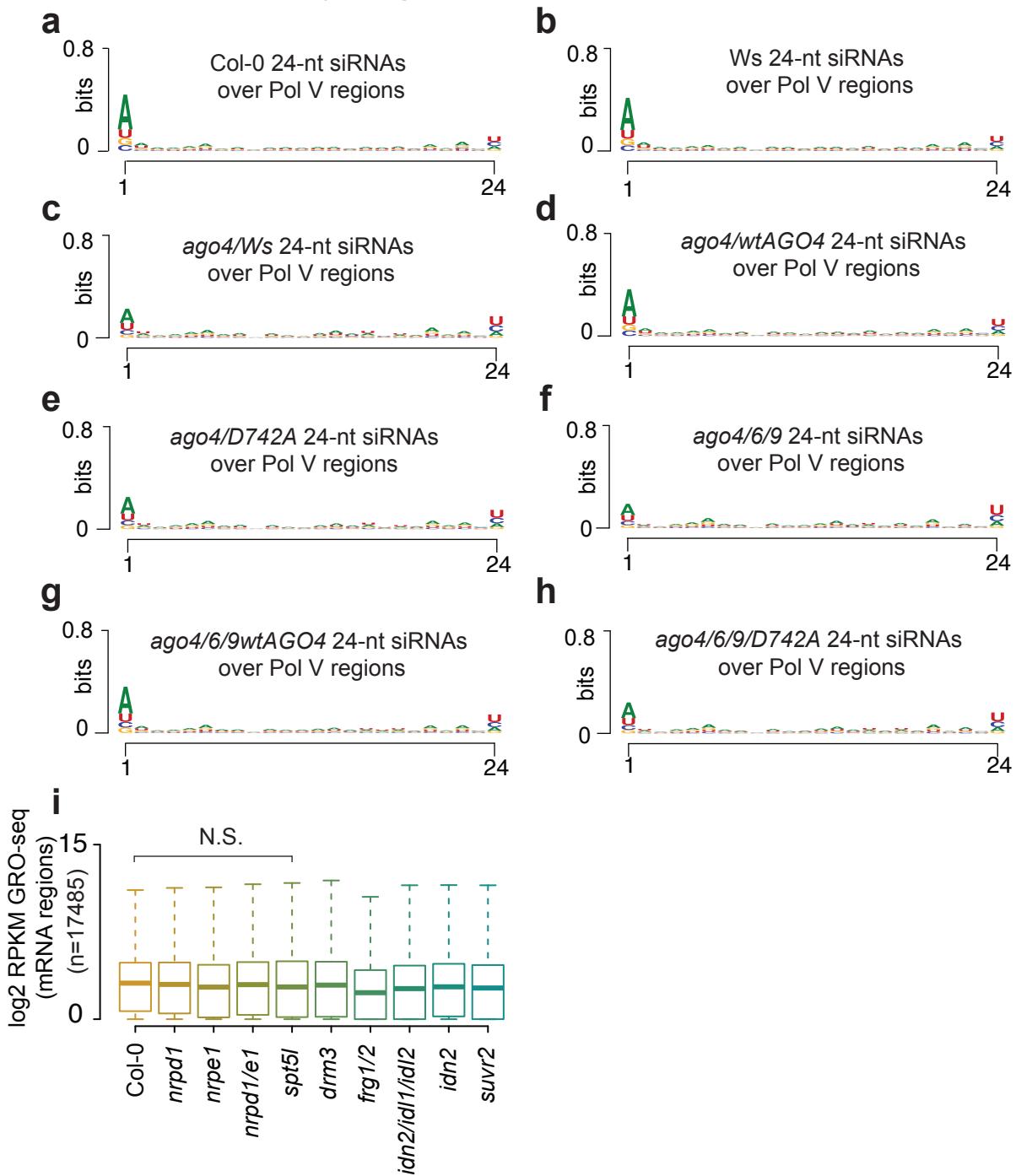
**Supplementary Figure 2.** Characterization of Pol IV/V-codependent sites and Pol IV-independent Pol V sites. **a,b**, Genome browser screenshot for Pol V ChIP-seq signals in Col-0 and GRO-seq signals in Col-0, *nrpe1*, *nRPD1*, and *nRPD1/e1* of a representative Pol IV/V-codependent site (**a**) and Pol IV-independent Pol V site (**b**). Plus (+) and Minus (-) indicate the strandness of GRO-seq signal. **c,d**, Heatmap of log<sub>2</sub> ratio of GRO-seq in Col-0 vs. *nrpe1*, GRO-seq in *nRPD1* vs. *nRPD1*, Pol V ChIP signals in Col-0, and Pol V ChIP-seq signals in *nRPD1* plotted over Pol IV/V-codependent sites (**c**) and Pol IV-independent Pol V sites (**d**). **e**, Boxplot of CG, CHG, and CHH methylation difference in *nRPD1* vs. Col-0. \*p-value < 0.05 (Welch Two Sample t-test). **f**, Normalized 24-nt siRNAs abundance in Col-0 over Pol IV/V-codependent sites and Pol IV-independent Pol V sites. \*p-value < 0.05 (Welch Two Sample t-test).

## Supplementary Fig.3



**Supplementary Figure 3.** Pol V transcripts with different lengths are sliced. **a**, Size distribution of nascent transcripts in *nrpd1* over Pol V-dependent regions. Replicates were merged for this plot. **b**, The percentage of U presented over genomic average at position 10 from the 5' ends of nascent transcripts captured with GRO-seq in six biological replicates for Col-0. **c,d**, The relative nucleotide bias of each position in the upstream and downstream 20-nt of nascent RNAs generated from the top 1,000 expressed annotated gene regions in Col-0 (**c**) and *nrpd1* (**d**). Replicates were merged for plot (**c-d**). **e-i**, The relative nucleotide bias of each position in the upstream and downstream 20-nt of nascent transcripts of 30- to 40-nt long (**e**), 40- to 50-nt long (**f**), 50- to 60-nt long (**g**), 60- to 70-nt long (**h**) and 70-nt and longer (**i**) captured in Col-0. Replicates were merged for plot (**e-i**).

## Supplementary Fig.4



**Supplementary Figure 4.** 24nt-siRNAs retain strong enrichment of A at position 1 for *ago4*, *ago4/6/9* mutant and *ago4* or *ago4/6/9* mutant expressing *wtAGO4* or *D742A*. **a-h**, The relative nucleotide bias of each position for 24-nt siRNAs over Pol V dependent regions in Col-0 (**a**), Ws (**b**), *ago4/Ws* (**c**), *ago4/AGO4* (**d**), *ago4/D742A* (**e**), *ago4/6/9* (**f**), *ago4/6/9/AGO4* (**g**) and *ago4/6/9/D742A* (**h**). **i**, Boxplot of normalized GRO-seq signals from top 1,000 expressed annotated gene in Col-0, *nrpd1*, *nrpe1*, *nrpd1/e1*, *spt5l*, *drm3*, *frg1/2*, *idn2/idl1/idl2*, *idn2*, and *suvr2*. N.S., not significant.